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Review

# Pharmacology and regulation of the neuronal dopamine transporter

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**Abstract**

The dopamine transporter, a member of the family of $Na^+$,$Cl^-$-dependent transporters, mediates uptake of dopamine into dopaminergic neurons by an electrogenic, $Na^+$− and $Cl^-$-transport-coupled mechanism. Dopamine and blockers of uptake such as cocaine probably bind to both shared and separate domains on the transporter, which can be influenced dramatically by the presence of cations. Regulation of the dopamine transporter occurs both by chronic occupancy with blocker and by acute effects of $D_2$ dopamine receptors or second messengers such as diacylglycerol (protein kinase C) and arachidonic acid. The dopamine transporter is involved in the uptake of toxins generating Parkinson's disease; it is also an important target for psychostimulant drugs, ligands for in vivo imaging and medications used for neurologic diseases involving changes in the dopamine system. 

*Keywords:* Dopamine transporter; WIN 35,428 binding; Arachidonic acid; Protein kinase C; Parkinson's disease; Drug abuse

## Contents



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## 1. Interest in the neuronal dopamine transporter

The Third Gaddum Memorial Lecture delivered 25 years ago by Iversen (1971) eloquently summarized the state of knowledge at that point in time. It covers uptake of catecholamines by postganglionic sympathetic neurons and sympathetic nerve terminals following the pioneering work of Axelrod and his colleagues, extraneuronal uptake of catecholamines ('Uptake$_2$') and brain neuronal uptake of norepinephrine, dopamine, serotonin, γ-aminobutyric acid (GABA), choline and glycine. The extraordinary insight into 'a general rule' associating high-affinity uptake with transmitter inactivation is truly impressive: all of these transport proteins discussed then (Iversen, 1971) are members of what we now know as the $Na^+$,$Cl^-$-dependent transporter family (see below). The use of subcellular fractionation techniques for brain preparations enriched in nerve endings made it possible to further characterize neuronal dopamine transport (Snyder and Coyle, 1969; Coyle and Snyder, 1969). Only later were radioligands developed for dopamine transporter binding assays analogous to those established previously for receptors (Hollenberg and Cuatrecasas, 1979). This development made it possible to study in detail the interactions between transmitters or transmitter-related compounds and transporters at the molecular level. The first transporter binding assays targeted the serotonin transporter with [$^3$H]imipramine (Langer et al., 1980) and [$^3$H]cocaine (Reith et al., 1980, 1983), soon followed by binding probes for the dopamine transporter such as [$^3$H]cocaine (Kennedy and Hanbauer, 1983; Calligaro and Eldefrawi, 1987), [$^3$H]mazindol (Javitch et al., 1984), [$^3$H]GBR 12935 (1-(2-(diphenyl methoxy)-ethyl)-4-(3-phenylpropyl)piperazine) (Berger et al., 1985) and others (for references, see Reith and Selmeci, 1992). Other inputs for increased interest in the dopamine transporter came from the discovery that this protein mediates the uptake of $MPP^+$ (1-methyl-4-phenylpyridinium ion), a highly neurotoxic oxidation product of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), into dopaminergic nerve terminals (see Langston, 1985). Ironically, this important finding, which led the way to a new animal model for Parkinson's disease, was precipitated by the development of severe parkinsonian symptoms in drug addicts self-administering what they believed to be synthetic heroin consisting of MPPP, the reverse ester of meperidine, rather than the synthetic by-product MPTP. Another important impetus for dopamine transporter research came from the realization of the importance of the dopaminergic system in brain reward systems (Wise, 1978) and the demonstration that the dopamine transporter is the initial target in the reinforcing action of psychostimulants such as cocaine (Ritz et al., 1987) and amphetamine (see Wise and Bozarth, 1987). Furthermore, imaging techniques (Carroll et al., 1995) building upon earlier ligand binding studies made it possible to tie dopamine transporter density or occupation to clinically relevant measures such as Parkinson's disease severity (Seibyl et al., 1995) or cocaine-induced euphoria (Malison et al., 1995). Finally, extraordinary progress was made in the field when the dopamine transporter was cloned (Shimada et al., 1991;

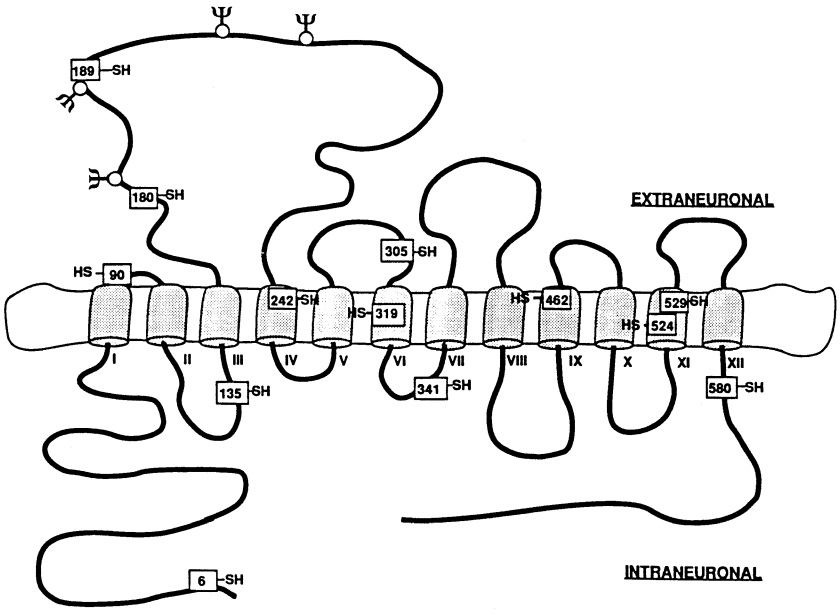


Fig. 1. Topographical representation of the dopamine transporter with the location of cysteinyl residues. □ – SH denotes a cysteine group; Ψ represents potential glycosylation sites. Reprinted by permission of John Wiley & Sons, Inc. from 'Mercuric chloride and *p*-chloromercuri-phenylsulfonate exert a biphasic effect on the binding of the stimulant [$^3$H]methylphenidate to the dopamine transporter' by M.M. Schweri, Synapse 16 (1994) 189, © 1994 John Wiley & Sons, Inc.

Kilty et al., 1991; Eshleman et al., 1995) subsequent to the norepinephrine transporter (Usdin et al., 1991) (see Section 2). The latest in vivo application of these techniques has led to the fascinating finding that dopamine transporter knockout mice do not respond to the effects of cocaine or amphetamine on locomotor activity and on dopamine uptake and release in contrast to wild-type mice (Giros et al., 1996).

## 2. Structure and function of the dopamine transporter

The dopamine transporter in neuronal plasma membranes is a member of the family of $Na^+$,$Cl^-$-dependent transporters which additionally include the transporter for norepinephrine (67% sequence identity), serotonin (49%), GABA (45%), glycine (41%), proline (44%), betaine (44%), taurine (42%) and creatine (43%) (Miller et al., 1997). The dopamine transporter cDNA codes for 620 amino acids, and hydropathy analysis suggests the presence of 12 transmembrane domains, with both the N- and C-terminus located in the cytoplasm (Shimada et al., 1991; Kilty et al., 1991; Eshleman et al., 1995) (Fig. 1). Translocation of dopamine by the transporter is accompanied by co-transport of probably two $Na^+$ ions and one $Cl^-$ ion (Rudnick, 1997); after release of dopamine inside, the transporter needs to be reoriented or 'returned', to the state with the substrate recognition site facing outward, and recent evidence indicates that outward transport of $K^+$ is not involved in this step (Gu and Rudnick, 1996), contrary to the situation for the serotonin transporter (Rudnick and Clark, 1993). If the reorientation step is rate-limiting in the transport cycle, it would explain the fact that dopamine is more potent in inhibiting its own translocation than in inhibiting binding of radiolabeled ligands to the dopamine transporter in striatal synaptosome-derived preparations ($K_m < K_i$) (see Zimanyi et al., 1989). The recent observations of the group of Rudnick (Gu et al., 1994) and ourselves (Reith et al., 1996b) suggest that this rate-limiting step depends on the system used for studying the dopamine transporter: in LLC-$PK_1$ cells or C6 glioma cells expressing the rat or human dopamine transporter, the $K_m$ and $K_i$ are quite comparable. It has been hypothesized that the characteristics of dopamine transport can be influenced by the level of glycosylation (Patel et al., 1993). The molecular weight of the human dopamine transporter protein in the caudate is 62–74 kDa, whereas that of the deglycosylated protein is approximately 50 kDa (Patel, 1997). There are three consensus sites (Asn-X-Ser/Thr with X not being Pro) for N-linked glycosylation in the extracellular loop between the 3rd and 4th transmembrane domain of the human dopamine transporter (Giros et al., 1992). It is likely that the stability and trafficking/targeting of the dopamine transporter greatly depend on the glycosylation state as shown for the glycine, norepinephrine and serotonin transporters (see Patel, 1997). The presence of cysteine residues in various locations in the dopamine transporter protein (Fig. 1) is consonant with the possibility of intra- or inter-molecular sulfur bridges, and supporting experimental evidence comes from molecular weight estimations by radiation inactivation indicating dimers (Berger et al., 1994) and tetramers (Milner et al., 1994). In analogy, evidence for oligomers including tetramers of membrane-bound serotonin transporters has been advanced recently (Jess et al., 1996). In consonance with the oligomeric structure of dopamine transporters, we observed that dopamine uptake inhibitors, when tested on [$^3$H]dopamine translocation and [$^3$H]WIN 35,428 (2β-carbomethoxy-3β-(4-fluorophenyl)[$^3$H]tropane, a centrally active cocaine congener) binding under identical conditions in rat striatal synaptosomal preparations, were approximately 2 times more potent in inhibiting uptake than in inhibiting binding (Xu et al., 1995), consonant with the idea of dopamine transporter dimers exhibiting receptor reserve characteristics when blocked by just one inhibitor molecule as opposed to binding measures reflecting the interaction between inhibitor and each transporter molecule within each dimer. The formation of dimers has also been invoked to explain the stimulation of [$^3$H]methylphenidate binding to the dopamine transporter in rat striatal membranes by reagents such as $HgCl_2$ that can cross-link inter-molecular sulfur bridges (Schweri, 1994).

## 3. Recognition sites for blockers and substrates on the dopamine transporter

In interacting with the dopamine transporter, substrates gain access to the opposite side of the plasma membrane by translocation whereas blockers are incapable of translocation. However, the initial step is universally thought of as a binding reaction between the ligand and the transporter protein, involving sites that 'recognize' certain classes of compounds relevant to transport (physiology) or blockade of transport (pharmacology). Classic uptake and binding experiments have generally indicated identical sites for the recognition of dopamine and uptake blockers such as cocaine, BTCP (*N*[1-(2-benzo[*b*]thiophenyl)cyclohexyl]piperidine) and GBR 12935 (Richelson and Pfenning, 1984; Shank et al., 1987; Krueger, 1990; Reith et al., 1992). However, other approaches have provided evidence for the involvement of distinct binding domains in addition to shared domains. For example, when membranes of rat striatum or cells expressing the human dopamine transporter were exposed to the alkylating action of *N*-ethylmaleimide, substrates and blockers offered differential protection (Johnson et al., 1992) even when differing affinities for the transporter were taken into account (Reith et al., 1996a; Xu et al., 1997). In addition, modeling of the interaction between

cocaine and dopamine uptake measured by classic radiolabeled tracer uptake (Wheeler et al., 1994) and by rotating disk voltammetry (McElvain and Schenk, 1992) in rat striatal suspensions suggested an uncompetitive action of cocaine involving $Na^+$ binding sites. Moreover, alterations in the dopamine transporter by site-directed mutagenesis indicated the importance of $Asp^{79}$, $Ser^{156}$ and $Ser^{159}$ in dopamine recognition, and of $Asp^{79}$ and $Tyr^{250}$ in WIN 35,428 binding (Kitayama et al., 1992; Davis et al., 1994), indicating both shared (in transmembrane domain 1) and separate (in transmembrane domains 4 and 7) sites of interaction for the substrate dopamine and the cocaine-like blocker WIN 35,428. Along similar lines, chimeras constructed of norepinephrine and dopamine transporter sequences indicated the importance of transmembrane domains 1–3 for both substrate and inhibitor interactions whereas transmembrane domains 5–8 appeared to be involved in the binding of inhibitors only (Giros et al., 1994; Buck and Amara, 1995). Of course, the possibility that some of the mutation or chimera effects are due to conformational changes introduced at sites distal from those involved in the actual binding interactions should always be considered. This consideration also applies to the protection experiments with alkylating agents discussed earlier. Thus, blockers may be better protectors against sulfhydryl reagents than substrates because they introduce conformational changes that make the cysteines inaccessible even though these residues may not themselves be involved in or near the binding pocket(s) of the blockers. Thermodynamic analysis indicated a change in entropy upon the binding of blockers but not substrates to the dopamine transporter (Bonnet et al., 1990), consonant with a conformational change for the binding of blockers but not substrates. However, the analysis assumes that the radioligand binds to the same site as the tested substrates, whereas most likely additional separate domains are involved which may be differentially affected by temperature changes.

## 4. Recognition sites for ions on the dopamine transporter

While $Na^+$, in the presence of other cations, stimulates dopamine transport (Zimanyi et al., 1989; Gu et al., 1994) and binding of radioligands such as [$^3$H]cocaine (Kennedy and Hanbauer, 1983; Reith et al., 1986; Calligaro and Eldefrawi, 1987) in striatal preparations containing dopamine transporters, cocaine has been reported to paradoxically prevent $Na^+$ from binding to the sites where $Na^+$ binds as a co-factor in dopamine translocation (McElvain and Schenk, 1992). In the absence of other cations, $Na^+$ has a biphasic effect on the uptake of [$^3$H]dopamine (Zimanyi et al., 1989) as well as the binding of [$^3$H]cocaine (Reith, 1988; Eshleman et al., 1993), or the cocaine analogs [$^3$H]WIN 35,428 (Reith and Coffey, 1993) and [$^{125}$I]RTI-121 (3β-(iodophenyl)tropan-2β-carboxylic acid isopropyl ester) (Chen et al., 1997), with low concentrations being stimulatory and high concentrations inhibitory. In contrast, $K^+$ primarily inhibits both dopamine transport and radioligand binding (Bonnet et al., 1988; Zimanyi et al., 1989; Reith and Coffey, 1993). The latter effect can be counteracted by increasing [$Na^+$] (Bonnet et al., 1988; Reith and Coffey, 1993), and this is also true for the inhibitory effect of other cations such as $Ca^{2+}$, $Mg^{2+}$, $Li^+$, $Tris^+$ and $choline^+$ on the binding of GBR 12783 (1-[2-(diphenylmethoxy) ethyl]-4-(3-phenyl-2-propenyl)piperazine) (Bonnet et al., 1988). These phenomena suggest intricate interactions between blockers such as cocaine or GBR 12783, dopamine and cations on the dopamine transporter. Indeed, site-directed mutagenesis has indicated the involvement of $Asp^{79}$ in the interaction with both dopamine and cocaine (Kitayama et al., 1992); this residue is located in a region related to ion dependence as indicated by transporter chimera studies (Giros et al., 1994; Buck and Amara, 1994). To take into account both stimulatory and inhibitory effects of $Na^+$, and to explain the interaction between blocker and $Na^+$ binding, we propose that, in addition to the site where $Na^+$ acts to stimulate cocaine binding, there is a cation site where $K^+$ and other cations, including $Na^+$ with lower affinity, act to mediate binding inhibition. Héron et al. (1996) also provide evidence for two cation sites where $Na^+$ and $K^+$ can interact to inhibit ligand binding, with one site postulated to overlap with the blocker binding domain. We postulate that this is the same site that overlaps with the cocaine binding domain where cations act to inhibit cocaine binding; this would explain the reduced potency of cocaine in inhibiting dopamine uptake at higher [$Na^+$] (Wheeler et al., 1994; McElvain and Schenk, 1992). Because dopamine and cocaine probably interact with both shared and distinct binding domains (Reith et al., 1992), dopamine recognition may not be under the same stimulatory and inhibitory control of $Na^+$ and other cations. These interactions may vary with temperature, and it will be important to assess them at the physiologically relevant temperature of 37°C with a radioligand that gives detectable binding levels such as [$^{125}$I]RTI-121 (our work in progress). Bonnet et al. (1994) have shown that cations interact with an inhibitory site located in the binding domain for GBR 12783-related compounds, most likely by recognizing −SH groups based on the rank order of potency of $CH_3Hg^+ > Cu^{2+} > Cd^{2+} > Zn^{2+} > Ni^{2+} = Mn^{2+} = Co^{2+}$ and the ability of dithiothreitol to reverse the binding inhibition. It remains to be determined whether this also applies to the binding of cocaine-related compounds. In this context, it should be mentioned that, although GBR 12783-related and cocaine-like compounds share common binding domains (Reith et al., 1992), separate domains are involved as well (Vaughan, 1995).

## 5. Active form of ligands for the dopamine transporter, electrogenicity and ionic currents

Dopamine, like norepinephrine and serotonin, can exist in the protonated or neutral (zwitterionic) form depending on the ambient pH. At neutral pH the bulk is in the protonated form, and this is still the case when the pH is raised to 8 or 9. The lack of pH dependency for the $K_m$ observed within this pH range for substrate translocation by the norepinephrine and serotonin transporter suggests that the protonated form is the active form for uptake (Rudnick, 1997). No such evidence is available in the case of the dopamine transporter. If the active form is protonated, and one transport cycle involves inward translocation of protonated dopamine, 2 $Na^+$ and 1 $Cl^-$, transport will be electrogenic (moving in two positive charges); if outward transport of 1 $K^+$ occurs as is the case for the serotonin (but not norepinephrine) transporter, electrogenicity will still apply (with the movement of one positive charge). However, recent evidence of Gu and Rudnick (1996) argues against the counter-transport of $K^+$. Consonant with electrogenicity, dopamine uptake by the cloned human dopamine transporter has been shown to depend on voltage (Zhu et al., 1995). Intriguing recent observations indicate that not only the dopamine transporter, but also the serotonin, norepinephrine and GABA transporters, catalyze, in addition to ion-coupled fluxes, leak currents that are not coupled to ionic movements (Rudnick, 1997; Povlock and Amara, 1997). It has been suggested that these uncoupled ionic currents reflect channel properties of transporters, consonant with viewing a transporter as a channel with two gates, of which only one is normally open at a time except for transient states, when both gates are open (leaking) (Rudnick, 1997). Another speculated role for these channel properties implicates membrane potential changes which could activate intracellular signaling mechanisms, somewhat like an autoreceptor (Povlock and Amara, 1997).

Until recently no information was available on what form is the active form of dopamine uptake blockers, many of which are weak bases that can be either protonated or neutral. Our recent work (Xu and Reith, 1996) addressed this issue for cocaine, which has a $pK_a$ value between 8.4 and 8.7 and is therefore largely in the positively charged form at neutral pH. For the local anesthetic action of cocaine at voltage-dependent $Na^+$ channels, this question was settled in favor of the positively charged form being active at the local anesthetic receptor, but the neutral form being crucial for penetrating the membrane on its way to the active site inside the hydrophilic vestibule in the channel (Ritchie and Greengard, 1966). For cocaine's action at the dopamine transporter in rat striatal membranes, pH-dependent changes in the binding of the cocaine analog [$^3$H]WIN 35,428 (displaying a higher affinity for the transporter than [$^3$H]cocaine yielding superior binding measures), were modeled in two ways, assuming either (1) pH does not affect the binding affinity of the ligand for the dopamine transporter, and the observed binding is a function only of the concentration of the active form; or (2) pH affects the binding affinity of the ligand for the transporter, and changes in this affinity as well as the concentration of the active form of the ligand in the medium contribute to the observed binding changes upon varying pH (Xu and Reith, 1996). For each model, predictions were formulated for the case that either the cation, neutral base, or both are the active form, and these predictions were compared with the experimental data. In addition, the pH-dependent changes of the binding of WIN 35,428, which can be both in the cationic and neutral form, were compared with those observed for cocaine methiodide, which is permanently cationic and benzocaine, which is permanently neutral. The surprising outcome was that both forms of WIN 35,428, cationic and neutral, were equally active in binding to the dopamine transporter (Xu and Reith, 1996). The development of the model underlying this conclusion is based on the assumption that WIN 35,428, cocaine methiodide and benzocaine bind to the same site at the dopamine transporter. Evidence in support of this comes from our recent experiments demonstrating an increase in the $K_d$ of

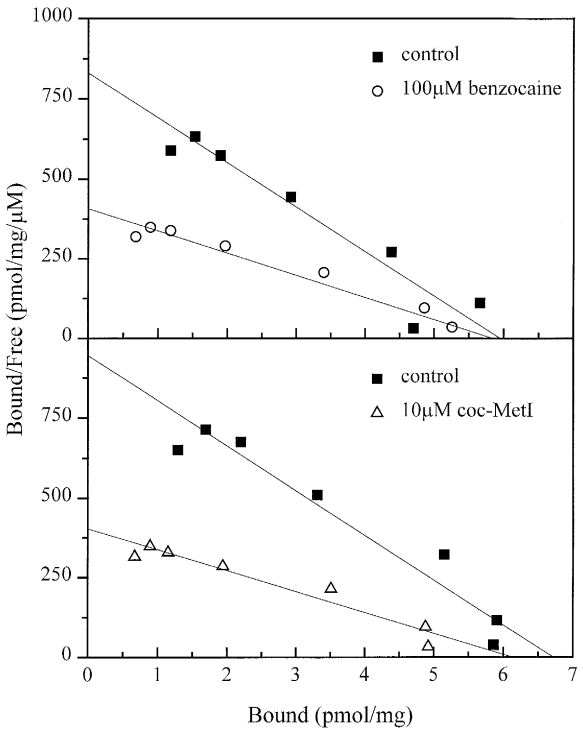


Fig. 2. Scatchard analysis of [$^3$H]WIN 35,428 binding to rat striatal membranes in the absence or presence of 100 μM benzocaine (upper panel) or 10 μM cocaine methiodide (coc-MetI) (lower panel). [$^3$H]WIN 35,428 was present at 2 nM and increasing concentrations of unlabeled WIN 35,428 were added up to 150 nM. Nonspecific binding was defined with 30 μM cocaine. The experimental conditions were as described previously (Xu and Reith, 1996). The straight lines represent the best fit chosen by the LIGAND program. Each panel shows a typical experiment replicated twice.

[$^3$H]WIN 35,428 binding to rat striatal membranes by the presence of 100 μM benzocaine (from $10 \pm 3$ to $18 \pm 2$ nM, average ± S.E. for three experiments) (Fig. 2, upper panel) or 10 μM cocaine methiodide (from $10 \pm 3$ to $21 \pm 3$ nM) without a change in the $B_{max}$ (6 pmol/mg of protein) (Fig. 2, lower panel). In other experiments, the $IC_{50}$ for benzocaine in inhibiting [$^3$H]WIN 35,428 binding was found to increase from $117 \pm 13$ to $256 \pm 23$ μM in the presence of 10 μM cocaine methiodide; the latter value was very close to the value of $236 \pm 20$ μM predicted by a one-site model for WIN 35,428, benzocaine and cocaine methiodide according to the analysis described previously (Reith et al., 1992). Most likely, a common domain is involved in the binding of all these compounds, impacted upon by pH. Alternatively, it cannot be ruled out that different domains play an additional role with pH impacting on such domains.

## 6. Regulation of the dopamine transporter

Changes in dopamine transport would be expected to impact on the temporal and spatial dynamics of dopamine neurotransmission by affecting dopamine inactivation. Alterations in dopamine transporter characteristics have been reported following chronic cocaine exposure in both animal models and in humans (for review, see Mash and Staley, 1997). The time since the last cocaine administration is an important factor in the end result. Thus, in vivo imaging in human cocaine addicts tends to show an initial increase in dopamine transporter density following acute cocaine binges followed by a normalization or decrease following longer withdrawal times (see Mash and Staley, 1997). Human cocaine overdose victims who died following a syndrome of excited delirium did not show an elevation of high-affinity radioligand binding to the dopamine transporter, perhaps explaining the agitation and paranoia associated with elevated dopamine in the absence of upregulated inactivation through transport (Mash and Staley, 1997). In other cases, observed changes in dopamine transporters are more likely due to changes in dopamine nerve terminal densities rather than regulation of transporter numbers per terminal. For instance, Parkinson's disease is an example where the observed reduction in dopamine transporters is due, at least in part, to the loss of nerve terminals carrying these transporters (Uhl and Kitayama, 1993).

There is also evidence for acute regulation of dopamine transport. Activation of $D_2$ dopamine receptors has been shown to increase striatal dopamine transport by an elevation in the $V_{max}$ (Meiergerd et al., 1993) (see dopamine terminals in the right portion of Fig. 3). Thus, increased synaptic dopamine levels may enhance clearance of dopamine in a feed-back manner. Other evidence focuses on the role of second-messenger systems. The cloned human and rat dopamine transporters contain consensus sites for phosphorylation by cAMP-dependent protein kinase and by protein kinase C in the third intracellular loop and in the N- and C-terminal domains (Giros and Caron, 1993). Indeed, cAMP has been shown to enhance uptake of dopamine into rat hypothalamic tuberoinfundibular dopamine neurons (Kadowaki et al., 1990), and activation of protein kinase C by phorbol esters decreases the dopamine transporting capability of the cloned rat (Kitayama et al., 1994) or human (Zhang et al., 1997) dopamine transporter. Recent information links phosphorylation of the dopamine transporter directly to uptake capability (Huff et al., 1997), although it is possible that phosphorylation at steps distal from the transporter is involved as well. Such complexities may play a role in the observed effects of increasing or decreasing phosphorylation on dopamine release through reversed transport that led to the conclusion that enhanced phosphorylation enhances rather than reduces dopamine transporter function (Giambalvo, 1992; Bugnon et al., 1995) (see also below). In addition to the action of the classic second messenger diacylglycerol on protein kinase C, other membrane phospholipids take part in activating protein kinase C such as arachidonic acid originating in the phospholipase $A_2$-induced phosphatidylcholine hydrolyis pathway (Asaoka et al., 1992). Thus, different pathways can converge upon protein kinase C to regulate dopamine transmission through changes in dopamine transporter function. In addition to the arachidonic acid-induced amplification of diacylglycerol-stimulated protein kinase C, the same signals that activate protein kinase C frequently cause the release of arachidonic acid through phospholipase $A_2$ activation (Nishizuka, 1995). Our recent work addressed the possibility that the effect of protein kinase C activation on dopamine transporter function was mediated by the release of arachidonic acid (Zhang and Reith, 1996). Indeed, as observed with protein kinase C activation, preincubation for 45–60 min with exogenously added arachidonic acid caused a reduction in human dopamine transporter measures, characterized by a decrease in both the $V_{max}$ of [$^3$H]dopamine uptake and the $B_{max}$ of [$^3$H]WIN 35,428 binding (Zhang and Reith, 1996). Enhancement of endogenous arachidonic acid by activating phospholipase $A_2$ or interfering with lipooxygenase (which breaks down arachidonic acid), also reduced transporter function. Because (i) staurosporine, an inhibitor of protein kinase C, did not counteract the arachidonic acid-induced decrease in dopamine uptake and (ii) bovine serum albumin, which binds arachidonic acid, attenuated the effect of arachidonic acid but not that of protein kinase C activation, it is likely that the inhibitory effects of arachidonic acid activators and those of protein kinase C activators on dopamine uptake are mediated by separate mechanisms. Curiously, shorter preincubations with arachidonic acid enhanced dopamine uptake by the human dopamine transporter, but this effect was not further characterized (Zhang and Reith, 1996). In comparison, L'hirondel et al. (1995) have re-

ported inhibition of dopamine uptake by rat striatal synaptosomes by arachidonic acid; at the same concentrations, dopamine release was stimulated, but not by transporter block because the release effect persisted in the presence of nomifensine. The releasing effect required protein kinase C activity, reminiscent of the phenomena reported in the two dopamine release studies discussed above (Giambalvo, 1992; Bugnon et al., 1995). As proposed by the group of Glowinsky (L'hirondel et al., 1995), the interesting possibility exists that glutamate, released from glutamate afferents in dopamine terminal areas in the brain, acts on glutamate receptors located on GABA cells to release arachidonic acid that in turn can act on dopamine terminals (see 'AA' in the middle of the right portion of Fig. 3). The resulting dopamine releasing activity, presumably mediated by protein kinase C, in combination with both stimulatory and inhibitory effects of arachidonic acid on dopamine uptake, would make complex regulation possible with a role for transmitters that act on G protein-coupled receptors altering phospholipase $A_2$ activity (see dopamine terminal in the middle of the right portion of Fig. 3).

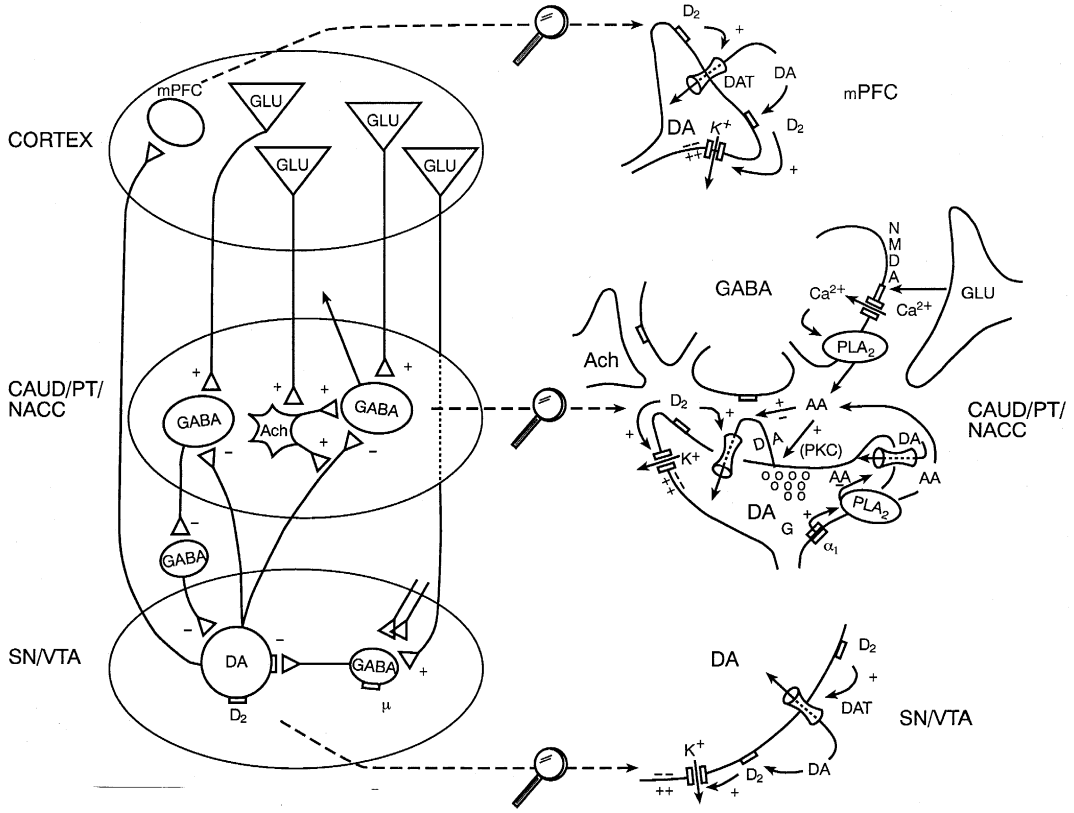


Fig. 3. Schematic representation of the involvement of the dopamine transporter (DAT) at various locations within dopaminergic pathways in the brain. (Left) Dopamine (DA) cells in the substantia nigra (SN) and ventral tegmental area (VTA) project to γ-aminobutyric acid (GABA) cells in the caudate (CAUD)/putamen (PT)/nucleus accumbens (NACC) and cells in the medial prefrontal cortex (mPFC). Feedback pathways originating in the CAUD/PT/NACC inhibit DA cell firing in SN/VTA. Glutamate (GLU)-ergic neurons in the cortex excite GABA cells and acetylcholine (Ach) interneurons in the CAUD/PT/NACC and GABA cells in the SN/VTA. μ-Opiate receptors on GABAergic interneurons in the SN/VTA serve to disinhibit DA cell firing. (Right) (arrows point to zoomed-in areas): In the mPFC, DA inactivation by the DAT is depicted as positively impacted upon by dopamine $D_2$ receptor activation as suggested for the striatum (Meiergerd et al., 1993) and the mechanism of which is not known (see Parsons et al., 1993). DA, acting on release regulating $D_2$ autoreceptors, perhaps coupled to $K^+$ channels assisting hyperpolarization as suggested for the striatum (Tanaka et al., 1992), inhibits DA release. The same phenomena are depicted in the DA terminals in the CAUD/PT/NACC. Additionally, arachidonic acid (AA) produced by phospholipase $A_2$ ($PLA_2$) activation following NMDA GLU receptor stimulation, stimulates DA release (involving protein kinase C) (L'hirondel et al., 1995) and has both stimulatory and inhibitory effects on DA uptake (observed by exogenous addition by Zhang and Reith, 1996). DA release is also depicted to be impacted upon by AA from $PLA_2$ activation through $\alpha_1$-adrenoceptors coupled to a pertussis toxin-sensitive G protein in analogy to findings in rat thyroid cells (Nishizuka, 1995); $\alpha_1$-adrenoceptors are known to reside on DA cell bodies (Grenhoff and Svensson, 1993) but their placement on the DA terminal is hypothetical; $PLA_2$ can also be activated by G-protein-coupled neurotransmitter receptors other than $\alpha_1$, or indirectly through receptors stimulating mitogen-activated protein kinases downstream from tyrosine kinases and protein kinase C (Nishizuka, 1995). Cytoplasmic AA generated by $PLA_2$ activation is depicted to primarily inhibit DA uptake because of the finding of inhibitory effects only upon endogenous $PLA_2$ activation (Zhang and Reith, 1996). In the SN/VTA (DA cell body areas), the DAT serves to diminish the action of DA on somatodendritic impulse-modulating receptors (see Chen and Reith, 1997), and is again depicted as being under positive control of dopamine $D_2$ receptors; $D_2$ receptors are also linked to hyperpolarization (Tanaka et al., 1992). There are little or no somatodendritic dopamine autoreceptors on cells projecting to the mPFC. The processes shown are only schematic and are not intended to be to scale. Synthesis-modulating autoreceptors in nerve terminals are not depicted here. For references on the brain circuitries, see Carlsson and Carlsson (1990), White (1990) and Di Chiara and North (1992).

## 7. Concluding remarks

The recent acceleration in transporter research, with the additional incorporation of molecular biological tools, has greatly advanced our knowledge about the structure, function and regulation of transporters. In the case of the dopamine transporter, this could have future practical applications. For instance, the delineation of transporter domains involved in interactions with blockers, as opposed to those interacting with dopamine, could lead to medications that block the effects of cocaine without themselves blocking dopamine uptake as adjuncts in the treatment of cocaine addiction (Kitayama et al., 1992; Xu et al., 1995). Elucidation of domains recognizing $MPP^+$ (Kitayama et al., 1993), as opposed to those involved in dopamine binding, could be beneficial for developing targeted treatment strategies for preventing the progression of Parkinson's disease; if uptake of putative neurotoxins is involved in the etiology of Parkinson's disease, medications that block neurotoxin uptake without interfering with dopamine transport would be desirable. Schizophrenia is generally believed to be associated with increased dopamine neurotransmission (Seeman and Niznik, 1990), and developments of compounds that accelerate rather than inhibit dopamine transport could conceivably be beneficial; with the current enhancement of knowledge concerning the basic functioning of dopamine transport, such compounds, as yet unknown, might become reality. Other neurologic diseases or symptomologies involving changes in the dopamine system such as Tourette's syndrome, attention deficit disorder, tardive dyskinesia and neuroleptic malignant syndrome, could also benefit from treatments targeted at the dopamine transporter. In addition, the possibility exists that inborn errors in the dopamine transporter protein or the regulation of its function contribute to the etiology of dopamine-related diseases. Recent studies along those lines have suggested an association between a variable number of tandem repeats polymorphism at the dopamine transporter locus and attention-deficit disorder (Cook Jr. et al., 1995) and cocaine-induced paranoia (Gelernter et al., 1994) but not schizophrenia (Bannon et al., 1995; Daniels et al., 1995). Finally, dopamine transporter ligands have been developed for in vivo imaging (Carroll et al., 1995), and are expected to aid in timely diagnosis of diseases involving changes in dopamine transporter density or their occupation by endogenous dopamine.

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